IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

		Atty. Docket: HOFFMAN9
In re Application of:	)	Conf. No.: 2518
Arnold HOFFMAN et al.	)	Art Unit: 1614
Appln. No.: 10/626,326	)	Examiner: J. D. Anderson
Filed: June 18, 2003	)	Washington, D.C.
For: REDDOX THERAPY FOR TUMORS	)	

DECLARATION UNDER 37 CFR §1.132

Honorable Commissioner for Patents
U.S. Patent and Trademark Office
Customer Service Window
Randolph Building, Mail Stop **Amendment**
401 Dulany Street
Alexandria, VA 22314

Sir:

I, Sanford R. SAMPSON, hereby declare and state as follows:

I am a professor at Bar-Ilan University in Israel and my educational and professional experience is presented in the curriculum vitae attached hereto.

The experiments described below were either conducted by me or under my supervision, and I can attest of my own personal knowledge that all the results reported hereby are true and accurate.

Effect of Redoxia compounds on 3T3 fibroblasts

3T3 fibroblasts, which are non-malignant cells that can be grown in culture, were treated with combinations of compounds obtained from Redoxia Israel, Ltd (herein referred to as "Redoxia" compounds), and according to the protocol (original concentrations as suggested by Redoxia) in Table 1 below.

Table 1

	DSF	BCNU	BSO	Curcumin
A	10^{-5}M	2×10^{-7}M		
B	10^{-5}M	2×10^{-7}M	5×10^{-3}M	
C	10^{-5}M	2×10^{-7}M	5×10^{-3}M	10^{-4}M

In addition, a second group of cells was untreated for the 48-hr period, and a third group was treated with a known inhibitor of cell proliferation (10^{-5}M Doxorubicin)

Cells were initially grown at 37°C in an atmosphere of 5% CO_2 and 100% humidity, and the medium was changed every other day and the day before the experiment. The day before treatment, cells were transferred to 96-well plates. The cells were in the logarithmic phase during the whole time of the experiment.

One day after seeding cells, compounds were added to the plates in the desired combination (see A, B and C in Table 1). Treatments were conducted for 48 hr followed by washing out of the compounds and replacement with fresh growth medium. All cells were examined 48 hr after the beginning of treatment and then 4 and 6 days after washout. Cells were also photographed 7 days after washout.

In this study, the viability assay, based on the metabolic activity measured in cell populations via incubation with a tetrazolium salt (e.g., MTT, XTT, WST-1) that is cleaved into a colored formazan product by metabolically active cells, was used. The absorbance, as determined on a spectrophotometer (ELISA Reader) is directly proportional to the number of metabolically active cells. Figures 1A-1C below show graphs of viability measurements made at 48 hours after treatment (Fig. 1A) and 4 days (Fig. 1B) and 6 days (Fig. 1C) after washout.

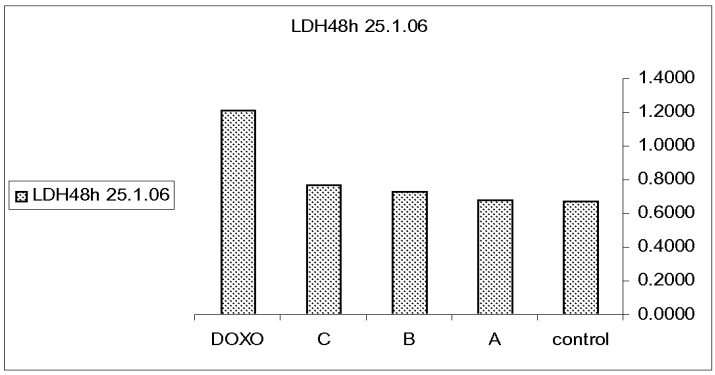

Fig. 1A

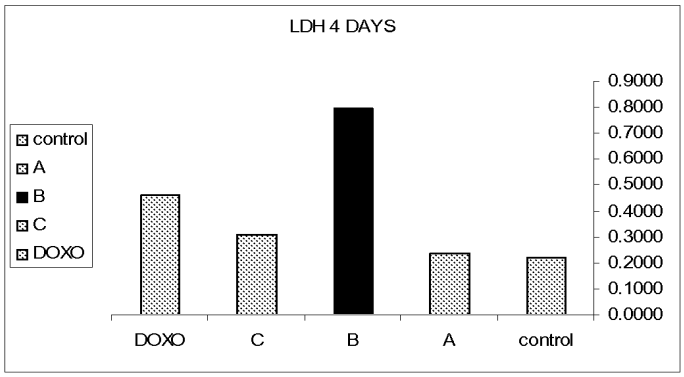

Fig. 1B

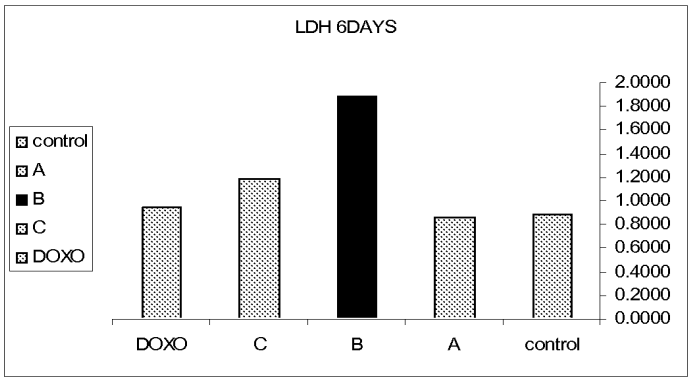

Fig. 1C

Viability measurements were made on doxorubicin-treated cells 4 and 6 days after washout. There were no viable cells in this group and, therefore, the values approached those obtained on the untreated control or compound-treated cells.

It is important to note that 4 and 6 days after washout, there was only a slight increase in the absorbance of the Group C treated cells. The other groups were not different from untreated. The exception is Group B, which for some reason gave extraordinarily high readings. We have no explanation for this aberrant behavior.

Figure 2 below shows photographs of 3T3 fibroblasts 2-weeks after seeding. Upper row: Control untreated cells. Middle row: cells were treated with either the Redoxia Compounds (left) or Doxorubicin (right) for 1 hr, washed and followed for 2-weeks. Lower row: cells were treated with either the Redoxia Compounds

(left) or Doxorubicin (right) for 2 hr, washed and followed for 2-weeks.

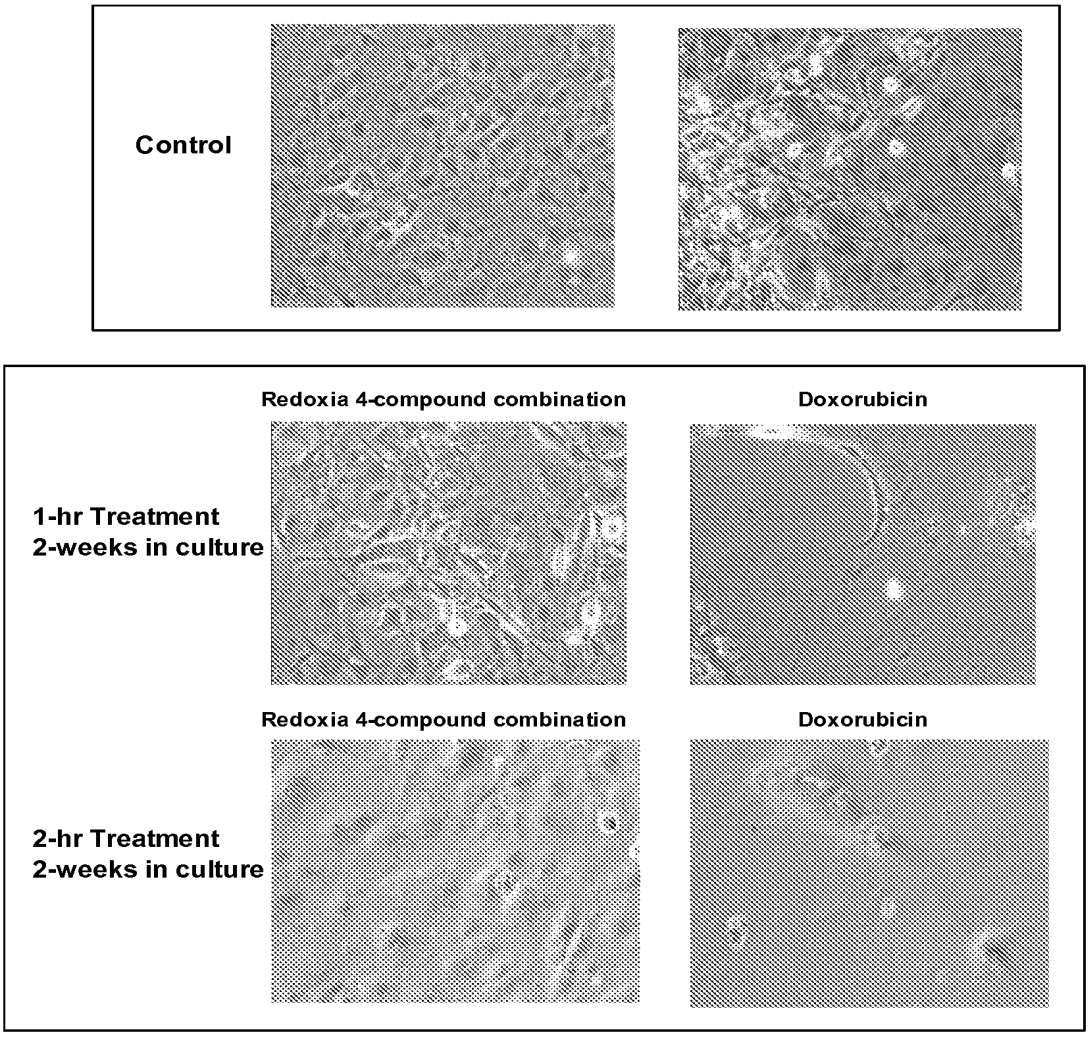

Figure 2

The results indicate that the Redoxia compounds interfere with proliferation of 3T3 fibroblasts but may not kill the cells, or may kill only a relative few. The cells appear to be able to resume proliferation after a delay. In contrast,

doxorubicin appears to kill virtually all the cells in the culture, and the cells do not resume proliferation.

In conclusion, no significant toxic effects of the Redoxia compounds on the 3T3 non-malignant cells could be detected with the combinations of Redoxia compounds for up to 7 days after washout. This is in contrast to doxorubicin which essentially killed all the cells.

Treatment of pancreatic and prostrate cancer cells

Cell proliferation in three types of pancreatic cancer cells, BXPC3, Colo and PAN 10.5 and in DU14S prostrate cancer cells, was determined with the use of a cell-proliferation kit, which measures the metabolic activity in cell populations via incubation with a tetrazolium salt (e.g. MIT, XIT, WST-1) that is cleaved into a colored formazan product by metabolically active cells. A combination of four Redoxia compounds/agents (10^{-5}M DSF, 2 x 10^{-7}M BCNU, 5 x 10^{-3}M BSO, 10^{-4}M Curcumin) were examined for their effectiveness. The positive control was treated with 10^{-5}M doxorubicin, a known inhibitor of cell proliferation, and the negative control is untreated cells. Cells were initially grown at 37°C in an atmosphere of 5% CO_2 and 100% humidity, and the medium was changed every other day and the day before the experiment. The day before treatment, cells were transferred to 96-well plates. The cells were in the logarithmic phase during the whole time of the experiment.

One day after seeding cells, compounds were added to the plates. Cells were treated for 48 hours and measured for cell survival. In these studies, the number of cells was determined by cell counting methods. The results for the prostate cancer cells and the three types of pancreatic cancer cells are presented below in Figures 3 (BXPC3), 4 (Colo), 5 (PAN 10.5) and 6 (DU145). All the treated cancer cells show dramatic reduction of cell proliferation compared to the negative control.

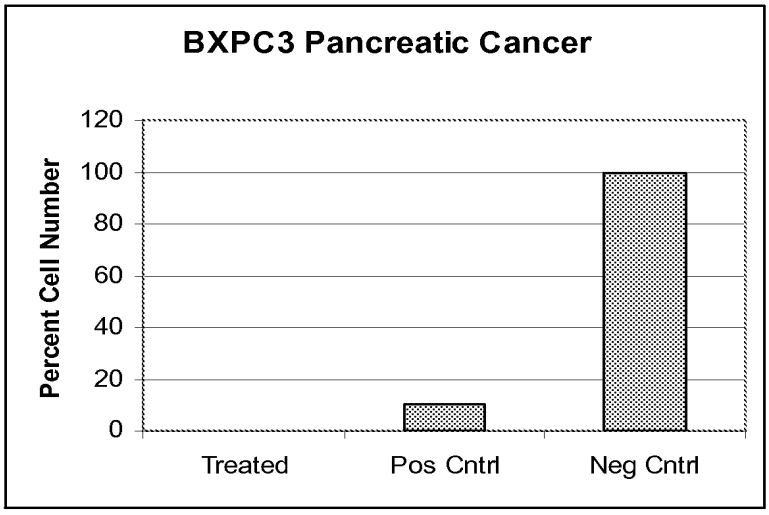

Fig. 3

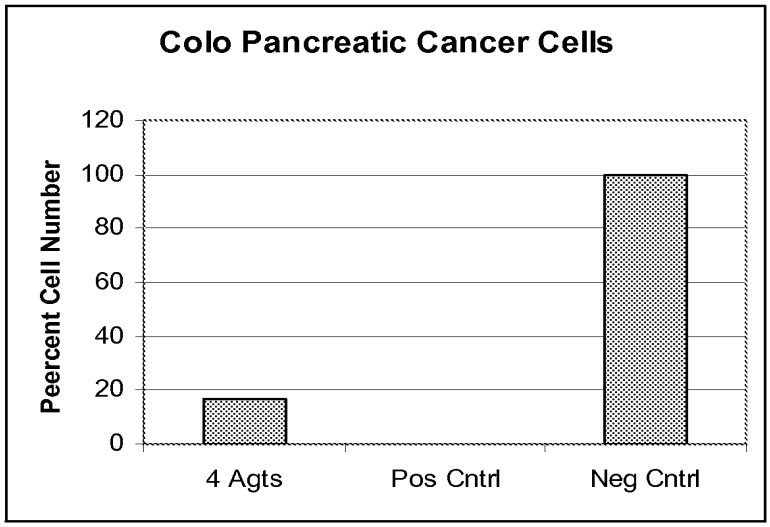
Colo Pancreatic Cancer Cells
Peercent Cell Number
120
100
80
60
40
20
0
4 Agts
Pos Cntrl
Neg Cntrl

Fig. 4

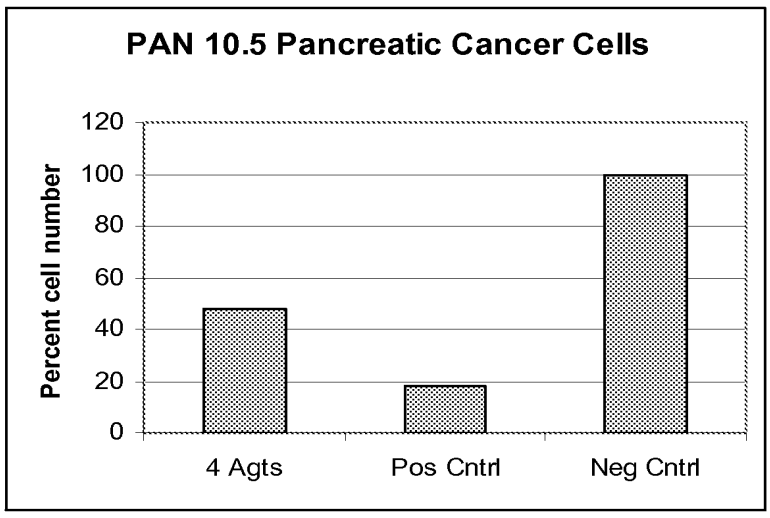
PAN 10.5 Pancreatic Cancer Cells
Percent cell number
120
100
80
60
40
20
0
4 Agts
Pos Cntrl
Neg Cntrl

Fig. 5

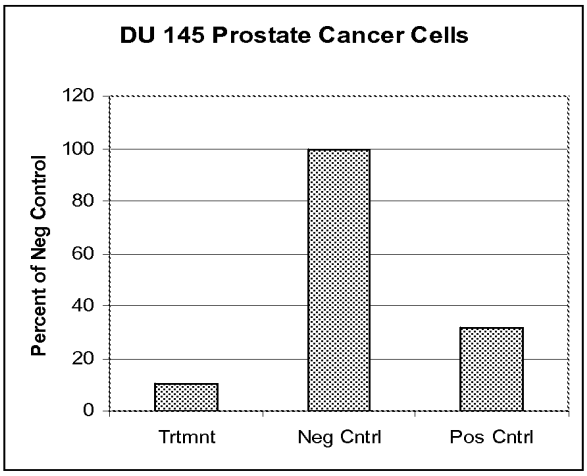

Fig. 6

Figure 7 below shows the effect of agents on mouse bladder tumor cells after 48 hour treatment. 2 Agnts = DSF+BCNU; 3 Agnts = 2 Agnts +BSO; 4 Agnts = 3 Agnts + curcumin. The concentrations used in each combination of agents were DSF 10^{-5}M, BCNU 2×10^{-7}M, BSO 5×10^{-3}M, curcumin 10^{-4}M. The combination of four compounds/agents in particular showed a dramatic decrease in cell survival (as percent of untreated control) compared to untreated control cells (medium).

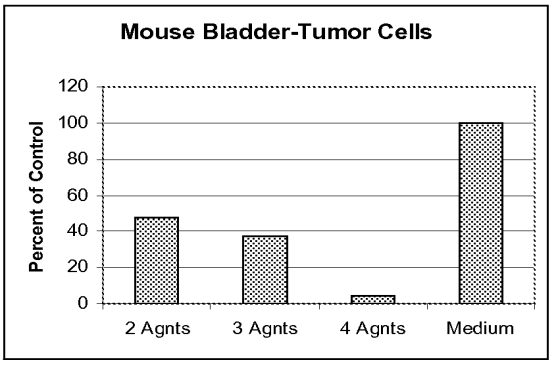

Fig. 7

The undersigned declares further that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

_____	_____
Date	Sanford R. SAMPSON

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A. ACADEMIC BACKGROUND AND TRAINING

Date:	**Institute**	**Degree**	**Area of specialization**
1959	University of California	B.A.	Physiology
1964	University of Utah	Ph.D.	Pharmacology
1959-1964	USPHS Pharmacology training grant, Dept. of Pharmacology, University of Utah College of Medicine	Predoctoral Fellow	Pharmacology/Physiology
1964-1966	Interdepartmental Institute for Training in the Neurologic and Behavioral Sciences, Dept. of Pharmacology, Albert Einstein College of Medicine	Postdoctoral Fellow	Neuropharmacology
1966-1969	Cardiovascular Research Institute, University of California, San Francisco Medical Center	Special Postdoctoral Fellow	Sensory Neurophysiology

B. PROFESSIONAL EXPERIENCE AND ACADEMIC APPOINTMENTS

Date:	Institute	Title	Research area
1979-present	Faculty of Life Sciences, Bar-Ilan University, Ramat-Gan, Israel	Professor	Cell Physiology and Biochemistry
2002-2004	Faculty of Life Sciences, Bar-Ilan University, Ramat-Gan, Israel	Dean	Cell Physiology and Biochemistry
1998-2002	Faculty of Life Sciences, Bar-Ilan University, Ramat-Gan, Israel	Department Head	Cell Physiology and Biochemistry
1994-2003	Academic Supervisory Committee, Optometry Studies Program, Bar-Ilan University, Ramat-Gan	Chairman	1994-2003
1990-1996	Otto Meyerhoff Center for Study of Drug-Receptor Interactions, Bar-Ilan University, Ramat-Gan, Israel	Head	Cellular Pathology
1988-	Louis Fisher Chair in Cellular Pathology, Bar-Ilan University, Ramat-Gan, Israel	Incumbent	Cellular Pathology
1985-	Bar-Ilan Health Science Continuing Education Center, Ramat-Gan, Israel	Academic Director	
1981-	Health Science Research Center, Department of Life Sciences, Bar-Ilan University, Ramat-Gan, Israel	Director	
1979-1982	Institute for Experimental Physiology and Surgery, Beilinson Hospital Medical Center, Petah Tikva, Israel	Associate Director	Cellular Neurophysiology
1978-1979	Neurobiology Department, Weizmann Institute of Science, Rehovot, Israel	Visiting Scientist	Cellular Neurophysiology
1974-1979	Department of Physiology, University of California, San Francisco	Associate Professor of Physiology in Residence	Sensory Neurophysiology
1973-1979	Cardiovascular Research Institute, University of California, San Francisco	Associate Staff	Sensory Neurophysiology
1971-1973	Cardiovascular Research Institute, University of California, San Francisco	Research Scientist	Sensory Neurophysiology
1971-1974	Department of Physiology, University of California, San Francisco	Assistant Professor of Physiology in Residence	Sensory Neurophysiology

1969-1971	National Heart & Lung Institute, Cardiovascular Research Institute	Special Fellowship	Sensory Neurophysiology
1969-1971	Department of Pharmacology, University of California, San Francisco	Assistant Professor in Residence	Pharmacology

RECENT PUBLICATIONS:

1. Braiman, L.W., Alt, A., Kuroki, T., Ohba, M., Bak, A., Tennenbaum, T. and **Sampson, S.R.** Protein kinase Cδ mediates insulin-induced glucose transport in primary cultures of rat skeletal muscle. Mol. Endocrinol. 13: 2002-2012, 1999.
2. Braiman, L.W., Alt, A., Kuroki, T., Ohba, M., Bak, A., Tennenbaum, T. and **Sampson, S.R.** Insulin induces specific interaction between insulin receptor and PKCd in primary cultured skeletal muscle. *Mol. Endocrinol.* **15**: 565-74, 2001.
3. Liu, Y-F., Paz, K., Alt, A., Kuroki, T., Ohba, M., Tennenbaum, T., **Sampson, S.R.**, LeRoith, D. and Zick, Y. Phosphorylation of insulin receptor substrate-1 (IRS-1) by PKCz negatively regulates IRS proteins function through a self-attenuated mechanism. *J Biol Chem*. **276**: 14459-14465, 2001.
4. Shefi-Frieedman, L., Wertheimer, E., Bak, A., Accili, D. and **Sampson, S.R.** Increased IGFR activity and glucose transport in cultured skeletal muscle from insulin receptor null mice. *Am J Physiol Endocrinol Metab*. **281**: E16-24, 2001.
5. Braiman, L.W., Alt, A., Kuroki, T., Ohba, M., Bak, A., Tennenbaum, T. and **Sampson, S.R.** Activation of protein kinase czeta induces serine phosphorylation of vamp2 in the glut4 compartment and increases glucose transport in skeletal muscle. *Mol Cell Biol*. **21**: 7852-7861, 2001.
6. Rosenzweig, T., Braiman, L., Bak, A., Alt, A., Kuroki, T. **Sampson, S.R.** Differential effects of tumor necrosis factor-a (TNF-a) on protein kinase C (PKC) isoforms alpha and delta mediate inhibition of insulin receptor signaling. *Diabetes* **51**: 1921-1930, 2002.
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8. Horovitz-Fried M., Cooper D.R., Patel N.A., Cipok M., Brand C., Bak A., Inbar A., Jacob A.I., **Sampson S.R.** Insulin rapidly upregulates protein kinase Cdelta gene expression in skeletal muscle. *Cell. Signal.* **18**: 183-193, 2006.
9. Horovitz-Fried M, Sampson SR. Involvement of PKCalpha in insulin-induced PKCdelta expression: Importance of SP-1 and NFkappaB transcription factors.

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10. Horovitz-Fried M, Jacob AI, Cooper DR, Sampson SR. Activation of the nuclear transcription factor SP-1 by insulin rapidly increases the expression of protein kinase C delta in skeletal muscle.Cell Signal. 2007 Mar;19(3):556-62.

11. Brand C, Cipok M, Attali V, Bak A, Sampson SR. Protein kinase Cdelta participates in insulin-induced activation of PKB via PDK1. Biochem Biophys Res Commun. 2006 Oct 27;349(3):954-62.

12. Sampson SR, Cooper DR. Specific protein kinase C isoforms as transducers and modulators of insulin signaling. Mol Genet Metab. 2006 Sep-Oct;89(1-2):32-47.. Review.

13. Cipok M, Aga-Mizrachi S, Bak A, Feurstein T, Steinhart R, Brodie C, Sampson SR. Protein kinase Calpha regulates insulin receptor signaling in skeletal muscle.
Biochem Biophys Res Commun. 2006 Jun 30;345(2):817-24

14. Sampson SR, Lupowitz Z, Braiman L, Zisapel N Role of protein kinase Calpha in melatonin signal transduction. Mol Cell Endocrinol. 2006 Jun 27;252(1-2):82-7

15. Rimler A, Jockers R, Lupowitz Z, Sampson SR, Zisapel N. Differential effects of melatonin and its downstream effector PKCalpha on subcellular localization of RGS proteins. J Pineal Res. 2006 Mar;40(2):144-52.

16. Heled Y, Dror Y, Moran DS, Rosenzweig T, Sampson SR, Epstein Y, Meyerovitch J. . Physical exercise increases the expression of TNFalpha and GLUT 1 in muscle tissue of diabetes prone Psammomys obesus. Life Sci. 2005 Oct 21;77(23):2977-85.

17. Rosenzweig T, Aga-Mizrachi S, Bak A, Sampson SR. Src tyrosine kinase regulates insulin-induced activation of protein kinase C (PKC) delta in skeletal muscle.Cell Signal. 2004 Nov;16(11):1299-308

18. Heled Y, Shapiro Y, Shani Y, Moran DS, Langzam L, Barash V, Sampson SR, Meyerovitch J. Physical exercise enhances hepatic insulin signaling and inhibits phosphoenolpyruvate carboxykinase activity in diabetes-prone Psammomys obesus. Metabolism. 2004 Jul;53(7):836-41

19. Koren R, Ben Meir D, Langzam L, Dekel Y, Konichezky M, Baniel J, Livne PM, Gal R, Sampson SR. Expression of protein kinase C isoenzymes in benign hyperplasia and carcinoma of prostate. Oncol Rep. 2004 Feb;11(2):321-6.

20. Heled Y, Shapiro Y, Shani Y, Moran DS, Langzam L, Braiman L, Sampson SR, Meyerovitch J. Physical exercise enhances protein kinase C delta activity and insulin receptor tyrosine phosphorylation in diabetes-prone psammomys obesus. Metabolism. 2003 Aug;52(8):1028-33.

21. Sharabani-Yosef O, Nir U, Sampson SR. Thyroid hormone up-regulates Na+/K+ pump alpha2 mRNA but not alpha2 protein isoform in cultured skeletal muscle. Biochim Biophys Acta. 2002 Nov 14;1573(2):183-8.

ABSTRACTS:

- ***Horovitz-Fried M.**, Rubinfeld H. and Sampson. S. R.* .Insulin Regulates Protein Kinase C delta (PKCδ) gene expression in skeletal muscle. Israel Endocrine Society - Annual meeting, Dec. 2002
- ***Horovitz-Fried M.**, Denise C., Sampson S. R.* Insulin Regulates Protein Kinase C delta Both Pre- and Post Transcriptionally. Israel Endocrine Society – Annual meeting, Dec. 2003
- ***Horovitz-Fried M.**, Cooper D. R. , Patel N., Cipok M., Brand C. and Sampson S.R.* Insulin Rapidly Increases Protein Kinase C delta Protein and RNA Expression in Skeletal Muscle. IXth International Symposium on Insulin Receptors And Insulin Action. Nice , October 14-17, 2004
- ***Horovitz-Fried M.**, Cooper D. R., , Patel N., Cipok M., Brand C. and Sampson S.R.* Insulin Rapidly Increases Protein Kinase C delta Protein and RNA Expression in Skeletal Muscle. Israel Endocrine Society – Annual meeting, Dec. 2004
- ***Horovitz-Fried M.**, Cooper D. R., , Patel N. and Sampson S.R.*
  Insulin Regulates Protein Kinase C (PKC) Expression in Skeletal Muscle. International congress of Endocrinology Lisbon, Portugal August 28 – September 2, 2004.
- ***Horovitz-Fried M.**, Cooper D. R., Patel N., Cipok M., Brand C., Bak A. and Sampson S.R.* Protein Kinase Cδ: An Immediate Early Response Gene in Insulin Signaling. IDA 2005
- ***Horovitz-Fried M.**, Brand C., Cipok M., Bak A., Patel N., Cooper D. R. and Sampson S. R.* Protein Kinase C delta Protein and RNA Expression are Rapidly Upregulated by Insulin in Skeletal Muscle. Annual Meeting of The Endocrine Society San-Diego , June 2005.
- *Inbar A., **Horovitz-Fried M.**, and Sampson.S.R.* Insulin Regulates Activity and Expression of Protein Kinase C Epsilon in Skeletal Muscle. Ilanit Eilat,2005.
- ***Horovitz-Fried, M.**, Jacob, A.I., Cooper, D.R. and Sampson, S.R.*
  SP-1 Transcription Factor Participates in Mediation of Insulin-Induced Transcription of Protein Kinase C Delta. The Endocrine Society Boston , June 2006.

- ***Horovitz-Fried M.****, Brutman-Barazani T and Sampson R. S.* Insulin Increases Nuclear PKCδ in L6 Skeletal Muscle. Israel Endocrine Society – Annual meeting, April. 2007.
- ***Horovitz-Fried M.****, Brutman-Barazani T and Sampson R. S.* Insulin Increases Nuclear PKCδ in L6 Skeletal Muscle. IDA 2007.
- *Brand C.*, ***Horovitz-Fried M*** *and Sampson R. S.* Insulin Induced Degradation of Protein Kinase C Delta Via The Ubiquitin-Proteasom Pathway. IDA 2007.
- *Brand C.*, ***Horovitz-Fried M.,*** *Brodie C and Sampson R. S.* Specific Tyrosine Phosphorylation Sites Regulate Insulin-Induced Degradation of Protein Kinase C Delta. . 67th Annual meeting of the American Diabetes Association Chicago, June 2007.